10,564,464
2-2-06

(5)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 July 2003 (24.07.2003)

PCT

(10) International Publication Number
WO 03/059360 A1

(51) International Patent Classification[7]: A61K 31/70

(21) International Application Number: PCT/US03/00834

(22) International Filing Date: 13 January 2003 (13.01.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/347,630 11 January 2002 (11.01.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/059360 A1

(54) **Title:** NOVEL CHOLESTEROL ABSORPTION LOWERING AGENTS

(57) **Abstract:** A process of preparing sitostanol glycosides in a form that is easily absorbed through the digestive tract, and methods of treating hypercholesterolemia by administering a composition which includes at least one glycoside or glycoside ester of a stanol glycoside are disclosed.

Novel Cholesterol Absorption Lowering Agents

Field of Invention

This invention relates to compositions and methods for reducing cholesterol absorption and serum cholesterol content in humans.

Background of the Invention

Phytosterols (plant sterols that are structurally similar to cholesterol) when ingested by humans, have been found to reduce cholesterol absorption and serum cholesterol levels, while not being absorbed themselves (Pollak, O.J. 1953. Reduction of blood cholesterol in man. Circulation 7:702-706, Ling, W.H. and P.J. H. Jones. 1995. Dietary phytosterols: A review of metabolism, benefits and side effects. Life Sciences 67:195-206, Kritchevsky, D. 1997. Phytosterols. Advances in Experimental Medicine and Biology 427:235-243). Lowering of circulating cholesterol and low-density lipoprotein cholesterol is an important part of a strategy to prevent and treat cardiovascular disease and especially coronary heart disease (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol. 1993. Summary of the second report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel II). JAMA 269:3015-3023). Cholesterol absorption is a critical component of whole body cholesterol metabolism. Cholesterol derived from the diet and also from endogenous biliary secretion enters the intestine and approximately 50% of the mixed intestinal load is absorbed (Bosner, M.S., Ostlund, R.E., Jr., Osofisan, O., Grosklos, J., Fritschle, C., Lange, L.G. 1993. Assessment of percent cholesterol absorption in humans with stable isotopes. J. Lipid Res. 34:1047-1064). The failure to absorb cholesterol quantitatively is therefore a key mechanism for the elimination of cholesterol from the body.

Most drugs currently available for treating high cholesterol levels have little or no effect on cholesterol absorption. For example, the potent new hydroxymethylglutaryl coenzyme A reductase inhibitors have a primary action to reduce cholesterol synthesis rather than increase cholesterol elimination. Bile acid sequestrants such as the ion-exchange resin cholestyramine act within the intestine but do not bind cholesterol and may actually increase cholesterol absorption when given repeatedly (McNamara, D.J., N.O. Davidson, P. Samuel, and E.H. Ahrens, Jr. 1980 Cholesterol absorption in man: effect of

administration of clofibrate and/or cholestyramine. J. Lipid Res. 21:1058-1064). Although when chronically administered, oral neomycin has been found to effectively reduce cholesterol absorption, neomycin is a potent, even toxic antibiotic (Samuel, P. 1979. Treatment of hypercholesterolemia with neomycin -- A time for reappraisal. N. Engl. J. Med. 301:595-597).

Since phytosterols are natural products which are non-toxic and inexpensive byproducts of food processing, they may be important in the treatment of individuals with mildly increased serum cholesterol or for the general population in food products or dietary supplements. The use of phytosterols could reduce the need for systemically absorbed drugs.

Despite their potential attractiveness, the usefulness of phytosterols has been limited by a large dosage requirement. For example, doses of 5-18 g sitosterol/day were found to reduce serum cholesterol by 16-20% (Farquhar, J.W. and M. Sokolow. 1958. Response of serum lipids and lipoproteins of man to beta-sitosterol and safflower oil. A long-term study. Circulation 17:890-899, Grundy, S.M., E.H. Ahrens, Jr., and J. Davignon. 19669. The interaction of cholesterol absorption and cholesterol synthesis in man. J. Lipid Res. 10:304-315). A dose-response study showed that 3-9 g of powdered sitosterol was needed to decrease serum cholesterol levels by 12% (Lees, A.M., H.Y.I Mok, R.S. Lees, M.A. McCluskey, and S. M. Grundy. 1977. Plant sterols as cholesterol-lowering agents: Clinical trials in patients with hypercholesterolemia and studies of sterol balance. Atherosclerosis 28:325-338). To reduce the required amount, recent experiments have used sitostanol, which appears to be more potent than other phytosterols and is non-absorbable (Sugano, N., H. Morioka, and I. Ikeda. 1977. A comparison of hypocholesterolemic activity of ß-sitosterol and ß-sitostanol in rats. J. Nutr. 107:2011-2019). In subjects with severe hypercholesterolemia, sitostanol at 1.5g/day reduced serum cholesterol by 15% (Heinemann, T., O. Leiss, and K. von Bergmann. 1986 Effect of low-dose sitostanol on serum cholesterol in patients with hypercholesterolemia. Atherosclerosis 61:392-396). However, sitostanol at 3 g/day had no effect in subjects with moderate hypercholesterolemia. (Denke, M.A. 1995. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. Am. J. Clin. Nutr. 61:392-396).

Several investigators have proposed ways to increase the solubility or bioavailability of phytosterols in order to make them more useful. Based on studies in rats and the finding that phytosterol esters are much more soluble in oil than the free sterols, it was proposed to use phytosterol esters in oil to lower cholesterol absorption (Mattson, F.H., F.A. Volphenhein, and B. A. Erickson. 1977. Effect of plant sterol esters on the absorption of dietary cholesterol. J. Nutr. 107:1139-1146). U.S. Patent 5,502,045 describes the use of sitostanol esters in oil for the treatment of hypercholesterolemia in humans. It was found that 2.8 g sitostanol/day given as a sitostanol ester in margarine reduced LDL cholesterol by 16% (Miettinen, T.A., P. Puska, H. Gylling, H. Vanhanen, an dE. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N. England J. Med. 333:1308-1312). However, the use of sitostanol esters dissolved in dietary fat has the disadvantage of requiring the administration of 23-50 g/day of dietary fat and of being 21% less effective at reducing cholesterol absorption in humans compared to the unesterified sterol (Mattson, F.H., S. M. Grundy, and J.R. Crouse. 1982. Optimizing the effect of plant sterols on cholesterol absorption in man. Am. J. Clin. Nutr. 35:697-700).

Additional work has focused on ways to improve the usefulness of unesterified phytosterols. International Patent Application WO 95/00158 describes a complex of sitosterol and the unabsorbable dietary fiber pectin and reports that it reduced serum cholesterol by 16.4% when given to hypercholesterolemic humans in a dose of 2.1 g/day. However, no measurements of an effect on cholesterol absorption were made and the complex was only about 50% soluble even at strongly alkaline pH suggesting that the bioavailability of the sitosterol component was limited. U.S. Patent 5,244,887 describes the use of stanols including sitostanol in food additives to reduce cholesterol absorption. For preparation of the additives, sitostanol is dissolved with an edible solubilizing agent such as triglyceride, an antioxidant such as tocopherol, and a dispersant such as lecithin, polysorbate 80, or sodium lauryl sulfate. However, no experimental data were given to guide one skilled in the art in the selection of the most effective components and their amounts or specific methods of preparation. Effectiveness in reducing cholesterol absorption was also not determined. The preferred embodiment consisted of 25% by weight stanols in vegetable oil, but the solubility of sterols in oil is only 2% (Jandacek, R.J., M.R. Webb, and F.H. Mattson. 1977. Effect of an aqueous phase on the solubility of cholesterol in an oil phase. J. Lipid Res. 18:203-210, Mattson, F.H., F.A. Volphenhein, and

B. A. Erickson. 1977. Effect of plant sterol esters on the absorption of dietary cholesterol. J. Nutr. 107:1139-1146) and that of sitostanol only 1% (Vanhanen, H.T., S. Blomqvist, C. Ehnholm, M. Hyvonen, M. Jauhianinen, I. Torstila, and T. A. Miettinen. 1993. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. J. Lipid Res. 34:1535-1544). Thus, it is not clear whether these food additives can even be made. U.S. Patent 5,118,671 describes the production of sitosterol-lecithin complexes as anti-inflammatory agents.

Cholesterol is absorbed from an intestinal micellar phase containing bile salts and phospholipids, which is in equilibrium with an oil phase inside the intestine. Delivery of phytosterol as a solid powder or aqueous suspension is not preferred because of the limited rate and extent of solubility in intestinal liquid phases. New phytosterol formulations are needed.

Summary Of The Invention

In one aspect, the present invention features formulations of stanol glycosides that are easily absorbed through the human digestive tract. Preferred glycosides include the glucosides, galactosides, maltosides, lactosides, or cellobiosides, e.g. ß-D-galactosides, ß-D-maltoosides, ß-D-lactosides or ß-D-cellobiosides. In a particularly preferred embodiment, the formulation comprises sitostanol-ß-D-glycoside, and campestanol-ß-D-glycoside. In another preferred embodiment, the stanol glycoside is dissolved or dispersed in a solubilizing macromolecule. Preferred solubilizing macromolecules include phospholipids, starch, modified starch, alphalized starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharides, disaccharides, polysaccharides, hydroxypropyl cellulose, methylcellulose, and lecithin. In particularly preferred formulations, the stanol glycoside or stanol glycoside ester has a particle size in the range of about one to about one hundred microns.

The formulation may be prepared from a solid residue remaining after removal of water or other solvents from a solution or suspension of said glycosides and the carrier or diluent. Typically, the stanol glycoside has a particle size of 1 - 100 micron. Stanol glycoside esters may also be used. These formulations are particularly useful as oral pharmaceutical compositions comprising an effective amount of the stanol glycoside and a pharmaceutically acceptable carrier or diluent.

Stanols are not water-soluble and, if they are not absorbed, they may be excreted after ingestion with little or no effect to lower cholesterol. The invention enhances bioavailability of stanol-glycosides by enhancing absorption in the intestine. The invention also avoids discomfort and other problems associated with oral administration of phytosterols – e.g., pure phytosterols pressed into one-gram tablets can create stomach disorders. Unmixed sitostanol powder may appear in stool samples from patients undergoing cholesterol turnover studies where sitostanol was given as a stool marker without any cholesterol-lowering effect.

According to the invention, sitostanol glycoside is delivered in a more soluble form without using oil or margarine as a vehicle avoiding the substantial disadvantage of administering oil or fat to a patient in need of cholesterol reduction. (Giving 3 g/day of sitostanol oleate in oil requires about 30 g oil or fat with 270 calories).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment and from the claims.

Detailed Description

We have prepared dispersions of Sitostanol in dispersions of liquids and solids. In addition we have prepared stanol derivatives like sitostanol glycosides and glycoside esters. The glycoside moiety enhances their absorption in the intestines and increases their bioavailability. Suitable sources of sterols as raw material include soybeans, wood, and apple presscake. The sterols are then converted to stanols by hydrogenation. Glycosylation of the stanols may be achieved by various techniques, e.g., by the general technique described by Vogel, Tetrahedron Ltrs. 26:1713 et. seq. (1985). Reactive monosaccharide derivatives used for glycosylation may be readily prepared or can be obtained commercially. Liposomes containing stanols-glycosides may be prepared by known techniques. Typical dosages according to the invention are from 0.1g - 10g/75kg patient. This dosage may be formulated in a powder or liquid and dispersed in hydroxypropyl methylcellulose, phospholipids, lecithin, or also in polysaccharides such as starch, guar gum, and pectin. The dispersion is inserted into a standard soft gel capsule or hard capsule. Various techniques are known to test the dosage in animals and humans.

Examples

1. Preparation of Sitostanol

Phytosterols from soybeans containing sitosterol campesterol and stigmasterol (30 gram) were dissolved in 400 ml of ethylacetate and poured into a 600 ml stainless steel pressure vessel. 2 gram of palladium on carbon (10% dispersion) was added. The pressure vessel was charged with hydrogen to a pressure of 1000 psi and magnetically stirred. After 2 hours no additional pressure drop was observed. After 24 hours the pressure was released and the content of the vessel was filtered to remove the catalyst. The solvent was evaporated on a rotary evaporator. The dry product was re-crystallized two times from hot ethanol. A sample dissolved in $CDCl_3$ analyzed by NMR showed the absence of double bonds.

2. Preparation of Sitostanol Glycosides

For the preparation of sitostanol glycosides, there are a number of reactive monosaccharide derivatives commercially available. Acetobromo- ß-D-glucopyranoside, and other conjugates can be prepared from similar derivatives, also available commercially, like the acetobromo derivatives of galactose, glucouronic acid, maltose, and fucose. A sample reaction is shown in Figure 1.

3. Preparation of Stanol-Glucoside- Methocell Dispersions 1:1.

Five hundred grams of soybean saturated stanols were dissolved in one and one-half liters of chloroform by warming and stirring. Five hundred grams of Methocell K100M PREM (Hydroxypropyl Methyl Cellulose) was dissolved in two liters of absolute ethanol by warming and stirring. The sitostanol glucoside solution was added to the methocell solution while both were warm with vigorous stirring. The material changed to a heavy paste upon cooling. The paste was spread out in a large glass tray and allowed to air dry for several days in the hood. The material was repeatedly broken up into small pieces as it dried. After several days the odor of the chloroform and ethanol were gone. The material was then dried under vacuum overnight. The material was added in small pieces to a blender and the material was reduced to a fine powder in small batches.

4. Testing of Sitostanol Glucoside in Animals for its Cholesterol-lowering Efficacy

Three concentrations of sitostanol glucoside were tested for cholesterol-lowering ability: 16% stanol by weight (MI-1), 33% stanol by weight (MI-2) and 66% stanol by weight (MI-3). All three concentrations were tested in the following screens and animal models: 1. Dissolution assay, 2. Hamster model, and 3. Dog model. All three analyses involved a standard control (without active ingredient) as well as a positive control (Benecol or the corresponding ester). The controls are highlighted within each data set. The resulting graphs, the supporting raw data, and our conclusions are presented below.

Dissolution Screen:

The dissolution assay is a primary screen to investigate the general ability of the formulation, stanol glucose dispersed in Hydroxypropyl Methyl Cellulose, to dissolve or disperse the associated stanol formulation in a simulated USP gastrointestinal fluid. Crystalline stanol assays at a value of 250 ug/ml (four hours) and 30 ug/ml (one hour). As one can see below, we obtained a fairly linear dose response (MI-C, MI-D, and MI-E) with the lower stanol concentration formulations providing the highest dissolution values. MI-A, MI-B, and MI-C demonstrate the reproducibility of the screen since these are virtually the same materials.

Hamster Screen:

The hamster study included two positive controls as well as a standard diet control. The two positive controls were crystalline stanol and stanol glucoside. All formulations/controls were administered in the chow through a process of grinding the formulation directly into the chow. Since grinding the formulations/controls into the chow itself contributes to the overall activity, this screen generally provides high levels of activity with less differentiation among the formulations tested. Six hamsters were used for each dose. As you can see below we did not obtain a linear response. MI-1 is the 1:5 ratio, MI-2 is the 1:2 ratio, and MI-3 is the 1-0.5 ratio.

Dog Screen:

The dog study included two controls, Benecol, the positive control, and the standard diet control. Formulations were administered in capsules and the Benecol administered via syringe. Dosing coincided with the morning meal. Six dogs were used for each dose. The results correlated well with the dissolution screen and unlike the hamsters, the dogs provided a nice dose response with no negative effect seen at the lowest stanol concentration formulation.

Conclusion:

There appears to be a direct correlation between concentration and activity. In all cases, except for the lowest concentration formulation in the hamster screen, there was a clear dose response with the most concentrated formulation providing the lowest unit activity. When compared directly with Benecol in the dog screen the most active dose (lowest concentration) appears to have activity similar to Benecol. The two more concentrated formulations have less activity. To obtain a 10% cholesterol lowering effect in the serum would therefore require 2.5-3 grams of Stanol Glucoside- Methocell if equivalent to Benecol without the intake of 30 g of fat in the Benecol.

Raw Data Behind the Graphs:

Hamster Study Data

Mean Cholesterol / Mean Sitostanol

% Level in diet	ID	Mean Cholesterol excretion ug/day	Mean Sitostanol excretion ug/day	Cholesterol/Sitosterol Ratio	Potency
1.0	SE	9639	43572	0.22	1.20
0.2	MI1	6765	15199	0.45	1.30
0.5	MI1	7211	37510	0.19	0.79
1	MI1	7976	58511	0.14	0.82
0.2	MI2	7746	10533	0.74	3.79
0.5	MI2	10513	25591	0.41	2.12
1	MI2	11853	44940	0.26	1.36
0.2	MI3	912	1538	0.59	2.58

0.5	MI3	1163	3141	0.37	1.61
1	MI3	1239	5031	0.25	1.07

Dog Study Data

ID	Mean Cholesterol Excretion ug/day	Potency
MI1	599301	1.02
MI2	428123	0.73
MI3	352003	0.60

Potency = Mean cholesterol excretion/Mean Benecol excretion

Hamster Potency = Mean cholesterol/Mean sitostanol ratio formulation group orMean cholesterol/Mean sitostanol ratio sitostanol group

What is claimed is:

1. A composition comprising a serum cholesterol lowering amount of a stanol glycoside and a dispersion agent.

2. A composition of claim 1, wherein the dispersion agent is selected from the group consisting of: a hydroxypropyl methylcellulose, starch, modified starch, dextrin, sodium starch phosphate, glucose, lactose, monosaccharide, disaccharide, polysaccharide, hydroxypropyl cellulose, methyl cellulose, phospholipid and lecithin.

3. A composition of claim 1 wherein the stanol glycoside is selected from the group consisting of a sitostanol glycoside, a sitostanol glycoside ester, a campestanol glycoside and a campestanol glycoside ester.

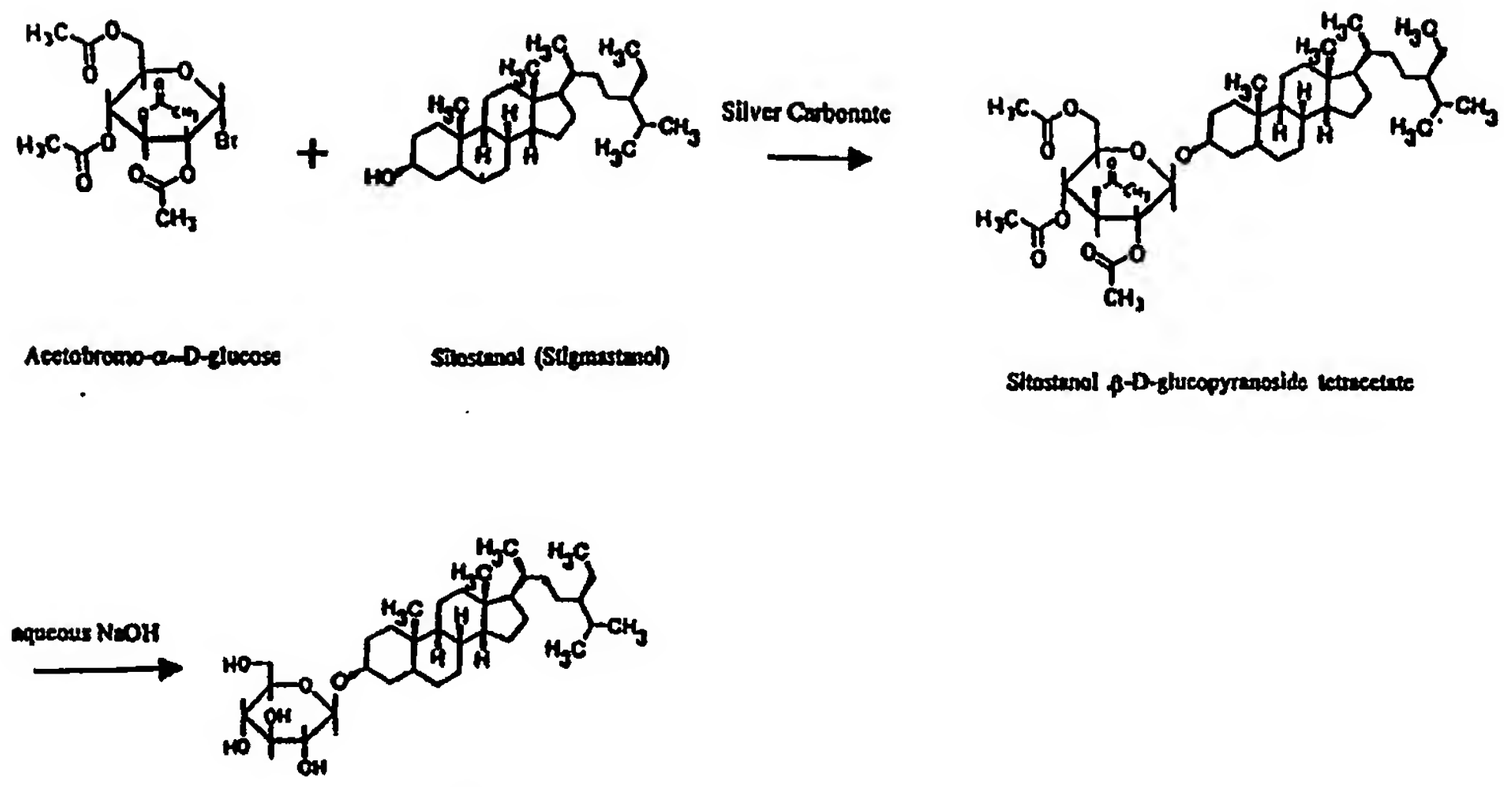
4. A composition of claim 1 wherein the glycoside is a glucoside, a galactoside, a maltoside, a lactoside or a cellobioside.

5. A composition of claim 2 wherein the dispersion agent is hydroxypropyl methylcellulose.

6. A composition of claim 1 wherein the stanol glycoside has a particle size in the range of about 1 - 100 microns.

7. A composition of claim 1, wherein the effective amount is in the range of about 0.1g to 10g.

8. A method for lowering the serum cholesterol level of a subject, comprising administering to the subject an effective amount of a stanol glycoside and a dispersion agent.



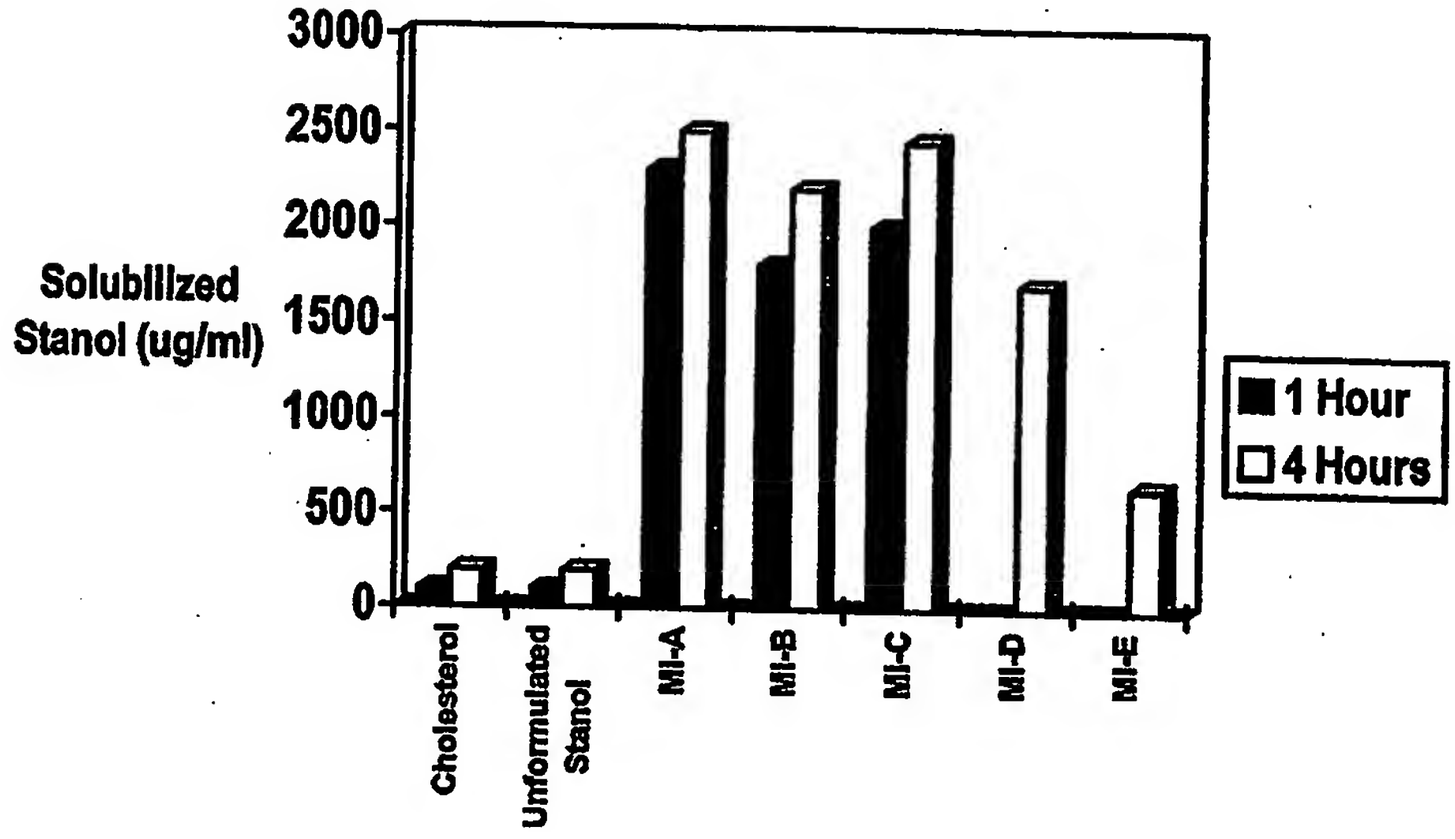
Acetobromo-α-D-glucose

Sitostanol (Stigmastanol)

Sitostanol β-D-glucopyranoside tetracetate

- Sitostanol β-D-glucopyranoside

Fig. 1



MI-A 1:5 Stanol hydroxy methylcellulose
MI-B 1:5 Stanol hydroxy methylcellulose
MI-C 1:5 Stanol hydroxy methylcellulose
MI-D 1:2 Stanol hydroxy methylcellulose
MI-E (1:0.5) Stanol hydroxy methylcellulose

Fig. 2

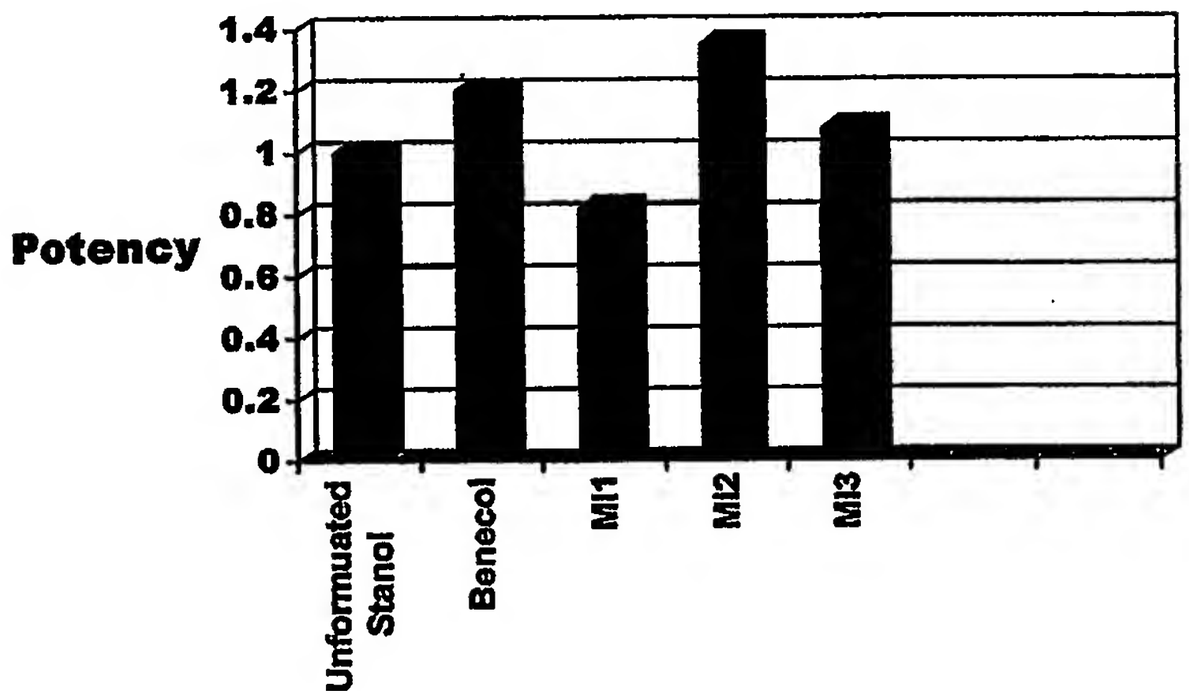

Hampster Screening Model Potency Data
(1.0% dose of the chow)
Potency
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Unformuated Stanol
Benecol
MI1
MI2
MI3

Fig. 3

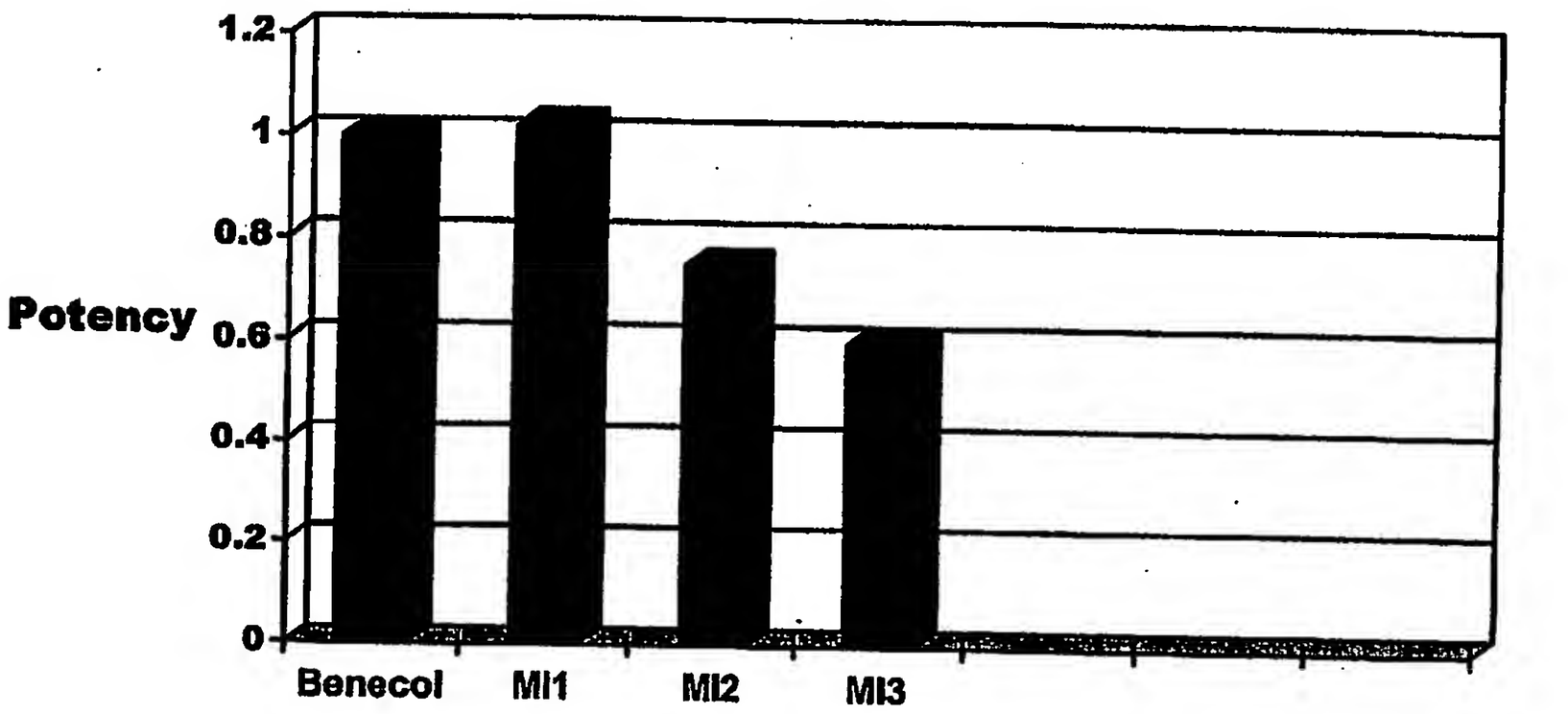

Dog Screening Model Potency Data
(0.2% dose of the chow)
Potency
1.2
1
0.8
0.6
0.4
0.2
0
Benecol
MI1
MI2
MI3

Fig. 4

Dog Screening Model Potency Data
(0.2% dose of the chow)

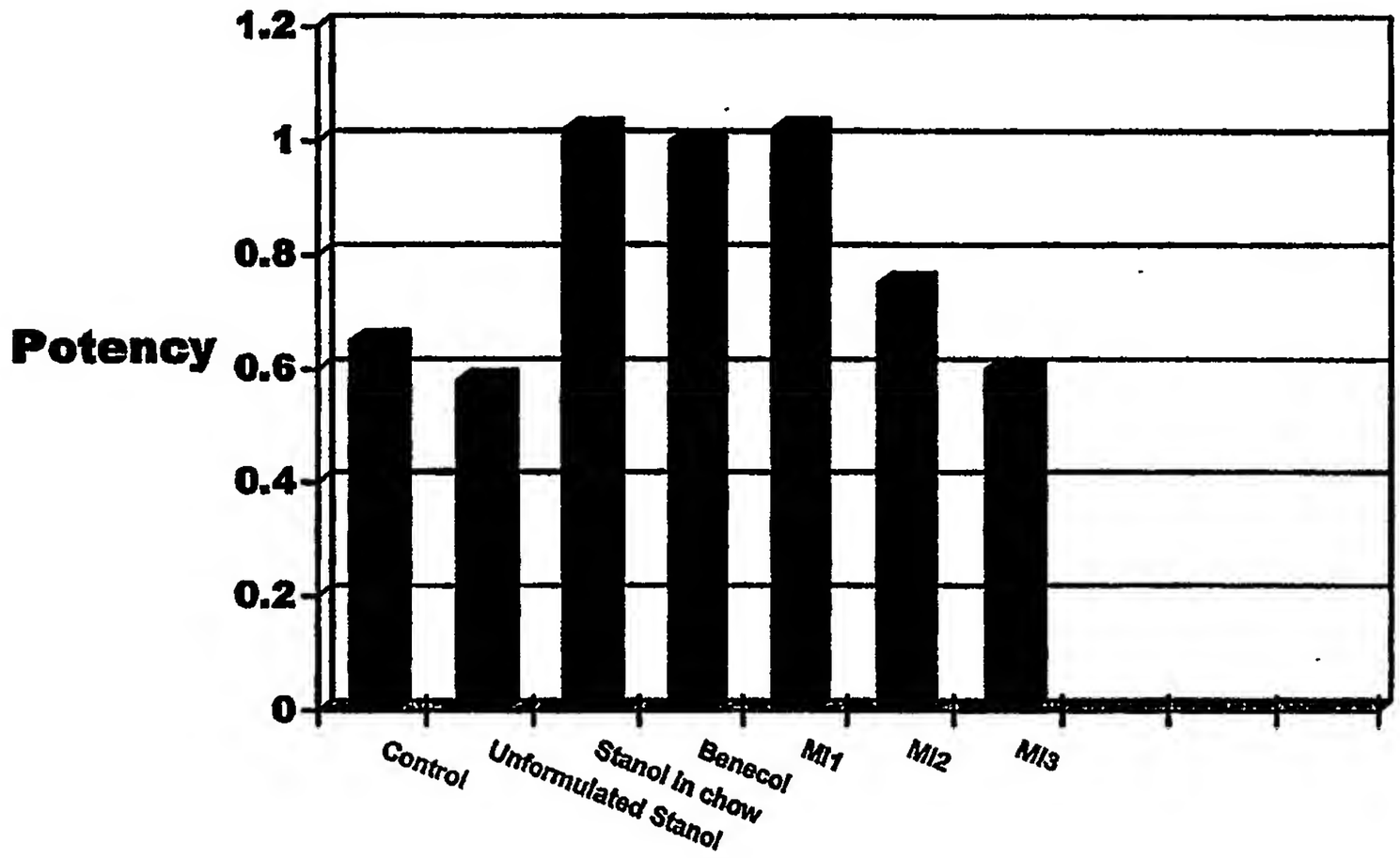


Fig. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/00834

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/70
US CL : 514/26
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/26

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,244,887 A (STRAUB) 14 September 1993 (14.09.1993), column 5, lines 56-64.	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 May 2003 (22.05.2003)	17 JUN 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Elli Peselev Telephone No. 703-308-1235

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